



Tumor microenvironment and immunology of ovarian cancer: 12th Biennial Rivkin Center Ovarian Cancer Research Symposium

Anirban K Mitra,¹ Yang Yang-Hartwich²

¹Medical Sciences Program,
Indiana University School of
Medicine, Bloomington, Indiana,
USA

²Obstetrics, Gynecology &
Reproductive Sciences, Yale
School of Medicine, New Haven,
Connecticut, USA

Correspondence to

Dr Anirban K Mitra, Medical
Sciences Program, Indiana
University School of Medicine,
Bloomington, IN 46202, USA;
anmitra@indiana.edu

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ABSTRACT

The 12th Biennial Ovarian Cancer Research Symposium organized by the Rivkin Center for Ovarian Cancer and the American Association for Cancer Research held on September 13–15, 2018 covered cutting edge and relevant research topics in ovarian cancer biology and therapy. Sessions included detection and prevention, genomics and molecular mechanisms, tumor microenvironment and immunology, novel therapeutics, and an education session. In this article we provide an overview of the key findings presented in the tumor microenvironment and immunology session.

INTRODUCTION

Ovarian cancer is the most lethal gynecologic malignancy and the fifth leading cause of cancer-related deaths among women in the USA.^{1,2} Recent developments in the field of ovarian cancer biology have uncovered several key findings that help us understand the disease better and provide us with new directions for improving patient outcome.³ Tumor microenvironment (TME) has emerged as an important area of interest. The TME includes the blood vessels, fibroblasts, immune cells, extracellular matrix (ECM), and all the signaling molecules surrounding the tumor.⁴ TME closely interacts with the tumor and mediates its initiation, progression, and metastasis.^{4–6} The vital TME components have become new therapeutic targets since they can profoundly affect patients' responses to treatments.⁷ Accumulating evidence suggests that the efficacy of chemotherapy and the promising technique of immunotherapy can be improved through the modulation of TME.⁸ Therefore, research interest in understanding the complexity and diversity of TME has exponentially increased in recent years. This aspect of ovarian cancer research was well represented in the 'Tumor Microenvironment and Immunology of Ovarian Cancer' session of the 12th Biennial Ovarian Cancer Research Symposium.

TUMOR MICROENVIRONMENT AND IMMUNOLOGY SESSION

The session included two invited speaker presentations followed by eight talks from selected abstracts. The first invited talk by **Dr Frances Balkwill** from

Barts Cancer Institute presented her laboratory's efforts to better understand ovarian cancer metastasis by first deconstructing metastasis in patient tumors and then developing experimental models based on the knowledge gained to accurately study the mechanism of regulation of metastatic colonization of the omentum. Using biopsies of metastasis from high-grade serous ovarian cancer (HGSOC) patients representing a spectrum of disease progression from marginal to aggressive, they profiled the dynamic interactions and changes in the tumor and stroma as the metastasis progressed. Extensive studies were performed using the same biopsy samples including gene expression, matrisome, ECM organization, biomechanical properties, cytokine/chemokine levels, and cellular profiles. The matrisome in humans consists of about 300 proteins that form the ECM, growth factors associated with the ECM, proteases and other ECM-modifying enzymes, and other ECM-associated proteins.⁹ Changes in the matrisome was a key feature identified, which could be correlated with prognosis and immune cell signatures that can themselves affect patient outcome. Through the reorganization of fibrillar collagens and the expression of glycoproteins and proteoglycans, the matrisome signature also determined the stiffness of the tumors. Moreover, there was a strong association between the number of α -smooth muscle actin and α -fibroblast-activated protein (FAP)-positive cancer associated fibroblasts (CAFs) and metastasis progression. Combining multiple different types of analysis on the same biopsies, and utilizing biopsies from patients exhibiting different extents of metastatic tumor progression, Dr Balkwill's group provided a comprehensive picture of the process. This complements the data from primary tumors provided by The Cancer Genome Atlas Program and can effectively form a platform for launching detailed studies deciphering the mechanism of metastatic progression in HGSOC as well as provide novel therapeutic targets to treat metastasis.

Dr Balkwill proceeded to describe her group's efforts to reconstruct the omentum in a petri dish to provide effective models to further study the mechanisms of the key factors identified by their multi-parameter analysis of ovarian cancer metastasis. Using



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HGSOC cells, fibroblasts, and adipocytes embedded in a collagen I gel, her group has developed tri-cultures representing the omental metastasis. Similarly, they have generated a quadri-culture model by including omental mesothelial cells. These models have been characterized for the expression of the matrix proteins identified in the deconstruction experiments, and transforming growth factor beta (TGF- β) was found to be a regulator of five of the six matrix molecules identified in the matrisome signature. Dr Balkwill also presented their characterization of mouse HGSOC models and presented data pointing towards the similarities in the ECM and immune subsets in the mouse and human tumors. Taken together, the models presented provide unique opportunities to study detailed mechanisms of metastatic colonization as well as drug discovery.

In the next invited talk, **Dr Ernst Lengyel** from the University of Chicago reported his group's recent findings on the role of cancer/testis antigen 45 (CT45) in increasing chemosensitivity of ovarian tumors. To study the role of the metastatic tumor proteome on the HGSOC patient outcome following chemotherapy, his group collaborated with Matthias Mann from the Max Planck Institute of Biochemistry. They developed a high-sensitivity, label-free proteomic mass spectrometry-based work-flow to analyze the proteome from formalin-fixed paraffin-embedded (FFPE) tumors. Using this approach they quantified more than 9000 proteins in chemosensitive and chemoresistant tumors. CT45 was the most upregulated in the chemosensitive tumors. It was found to affect DNA damage repair pathways by regulating protein phosphatase 4. The expression of CT45 is suppressed in the normal ovary by DNA methylation and is significantly upregulated in ovarian tumors through the loss of methylation. By combining immunopeptidomics and mass spectrometry, Dr Lengyel identified the role of CT45 as a cancer antigen presented on human leucocyte antigen class I receptors. These CT45 peptides were found to be potent in activating patient-derived cytotoxic T cells and inducing cancer cell killing. This novel comprehensive proteomics strategy incorporating proteome quantification, phosphoproteomics, interactome studies, and immunopeptidomics was effective in analyzing achieved FFPE tumor samples and identifying a unique biomarker, which is particularly relevant to long-term patient survival and immunotherapy.

Dr Lengyel also described the reciprocal signaling between CAFs and ovarian cancer cells identified by quantitative label-free mass spectrometry-based phosphoproteomics in co-cultures of CAFs and ovarian cancer cells. His group had previously shown that the metastasizing ovarian cancer cells utilized lipids released from the adipocytes in the omentum to drive their growth. His present findings demonstrate that the reciprocal signaling between CAFs and the cancer cells help the cancer cells switch their metabolism towards utilizing glycogen once the fat reserves are depleted. The activation of p38 mitogen-activated protein kinase signaling in the CAFs by the cancer cells resulted in the increased secretion of the cytokines interleukin 6 (IL-6), C-X-C motif chemokine ligand 10 (CXCL10), and C-C motif chemokine ligand 5 (CCL5). The secreted cytokines activated glycogenolysis in the cancer cells in a paracrine manner by activating phosphoglucomutase 1. Glycogen phosphorylase inhibition reduced metastasis in mice indicating that blocking glycogen mobilization could be a potentially effective therapeutic strategy to treat metastasis.

Dr Laurie Ailles from the University of Toronto presented her group's recent findings on the differential gene expression profiles

of ovarian cancer CAFs and cancer cells. CAFs could be subdivided into two groups based on the expression levels of FAP. FAP high and FAP low CAFs had distinct transcriptional programs, and analysis of The Cancer Genome Atlas data demonstrated a shorter progression-free and overall survival in FAP high patients. FAP high CAFs were functionally distinct from FAP low CAFs, with the FAP high CAFs having a greater ability to promote cancer cell invasion in vitro and tumor growth in mice. It would be interesting to analyze the matrisome signature described by Dr Balkwill in these FAP high and FAP low CAFs. The heterogeneity and potential functions of the subsets of CAFs would be relevant in future strategies involving targeting of the tumor stroma. **Dr Katherine Fuh** from Washington University presented her group's findings on the role of discoidin domain receptor 2 (DDR2) expression in fibroblasts in promoting ovarian cancer metastasis. Inhibition or silencing DDR2 in fibroblasts decreased cancer invasion and mesothelial cell clearance as well as decreased collagen staining (trichrome) intensity and quantity in tumors. This suggests the possibility of targeting the stromal DDR2 as a potential therapeutic option, and again calls for the analysis of the secretome of the fibroblasts and the cancer cells regulated by DDR2. **Dr Sara Zanivan** from the University of Glasgow reported the role of oxidoreductase chloride intracellular channel protein 3 (CLIC3) as a secreted factor and key contributor of tumor-stromal interaction. Through the mass spectrometry-proteomic comparative analysis of CAFs and their normal counterparts, CLIC3 was identified as the most upregulated and the most deposited in ECM. Further analysis revealed that the abundant CLIC3 in tumors was secreted by both stromal and cancer cells and could activate tissue transglutaminase-2 to promote blood vessel growth and increase tumor invasiveness. Their findings suggest a new mechanism of TME factor-mediated tumor invasion.

Understanding the complexity and diversity of immune cells in the TME has become critical for maximizing the clinical benefits from immunotherapy. **Dr Alan D'Andrea's** group at Harvard Medical School applied a novel high-multiplex tissue cyclic immunofluorescence (t-CyclIF) platform to understanding the dynamics between DNA damage in cancer cells and the immune context in HGSOC TME. This platform quantifies the expression of 60 antigens at single cell resolution. Data from over 10^5 cells showed distinct cell compositions in the TME of BRCA1/2 mutant and homologous recombination wild-type HGSOCs. On one hand, tumors with high programmed cell death protein 1 (PD-1) and its ligand (PD-L1) expression have high infiltration of CD1c+dendritic cells, which indicates the suppression of antigen presenting pathway and that these tumors are likely to respond to immune checkpoint blockade. Conversely, a subset of tumors with high levels of DNA damage show active interferon signaling and high CD8+ cytotoxic T-cell infiltration suggesting an immunogenic phenotype in this subset of HGSOCs. The application of new technologies like t-CyclIF will contribute to the development of rational combination therapies and predictive biomarkers for DNA damaging agents and immune checkpoint blockade. **Dr Pamela Kreeger's** group at the University of Wisconsin-Madison analyzed the secretome of macrophages and identified fms-related tyrosine kinase 3 ligand, heparin-binding epidermal growth factor, IL-6, IL-8, and leptin to be associated with tumor spheroid spreading in a macrophage-HGSOC spheroid co-culture using a 35-cytokine-multiplex assay. Although each ovarian cancer cell line (eg, OVCA3, OVCA433, and OV90) responded to a different

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set of cytokines secreted by macrophages, they utilized a common signaling pathway to regulate spheroid spreading, which is the Janus kinase 2/signal transducers and activators of transcription 3 (JAK2/STAT3) activation leading to matrix metalloproteinase-9 (MMP-9)-promoted tumor spreading. These findings suggest that multiple macrophage-secreted factors drive the tumor metastasis in HGSOC patients. However, they may share the same downstream signaling pathways, such as JAK2/STAT3/MMP-9. The identification of this molecular mechanism indicates the possibility of controlling tumor metastasis in a broad group of patients by targeting the main common signaling pathways for macrophage-tumor interaction. **Dr Ronny Drapkin's** group at the University of Pennsylvania revealed the role of ring finger protein 20 (RNF20)/histone H2B monoubiquitylation (H2Bub1) loss as an early event in HGSOC that modulate the immune signaling pathways during tumor initiation. H2Bub1 is an epigenetic regulator and tumor suppressor that is lost in serous tubal intraepithelial carcinomas (STICs) and HGSOCs. Ubiquitin ligase RNF20 catalyzes H2Bub1. Their data demonstrated that the inhibition of RNF20 altered immune signaling pathways and led to increased cell migration and clonogenic growth. The loss of RNF20/H2Bub1 functions is possibly responsible for the early oncogenic phenotype in STICs.

Angiogenesis is another important therapeutic target in the TME. **Dr Anil Sood's** group in the University of Texas MD Anderson Cancer Center identified a new target for overcoming the resistance to anti-angiogenic therapy. p130cas (Crk-associated substrate) is a central regulator of focal adhesion kinase (FAK)/Src-mediated angiogenesis. Their data showed that p130cas was highly expressed in the tumor-associated vascular endothelium. Ablation of p130cas gene or inhibition of its expression in mouse models of ovarian cancer increased the sensitivity to anti-vascular endothelial growth factor antibody treatment and inhibited tumor growth through autophagy-regulated cell death in endothelial cells. They have generated nanoparticle-delivered peptide antagonist to p130cas as a targeted therapeutic agent. The antagonist's clinical efficacy and mechanism of action are under evaluation.

It is also very exciting that new imaging technologies have been applied to the quantitative assessment of the architectural features in the TME of ovarian cancer. **Dr Paul Campagnola's** group at the University of Wisconsin-Madison used collagen-specific sensitive second harmonic generation imaging microscopy, 3D texture analysis, and machine learning to extract textural features and build models of the ECM in the ovarian cancer TME. They generated models for normal stroma, high-risk stroma, benign tumor, high-grade serous, low-grade serous, and endometrioid carcinoma. By examining the collagen alterations, they developed quantitative biomarkers for assessing the increased collagen concentration and the changes of alignment of collagen molecules within fibrils and fibers. Their data indicate that combining macro/supramolecular probes and the fiber morphology classification improves our understanding of TME evolution in ovarian cancer and the role of ECM alteration in disease etiology. This novel approach has the potential to be developed into new prognostic and diagnostic methods.

The poster session included very interesting presentations covering epigenetic modulators, non-coding RNAs, tumor immunology, in vitro models, cancer stem cells, novel therapeutics, and so on. Coffman reported that ovarian cancer cells mediate EZH2 induction and epigenetic reprogramming to convert mesenchymal

stem cells into carcinoma-associated mesenchymal stem cells. Zhang demonstrated that a combination of histone deacetylase 6 inhibition with PD-L1 checkpoint blockade could be a potential strategy to treat ARID1A-mutated clear cell ovarian cancer. Inhibition of nuclear factor- κ B activity in macrophages and potentially other cells in the ovarian TME was shown to inhibit tumor progression by Yull. Other studies included association of decreased let-7 with stemness, the role of long non-coding RNAs in metabolism, autophagy, or immune response, and a glycosylation-dependent mechanism involving Sox2 that drives a cancer stem cell phenotype. Posters covering tumor immunology demonstrated that the inositol-requiring enzyme 1/X box binding protein 1 arm of the endoplasmic reticulum stress response pathway in dendritic cells was necessary for accelerated ovarian cancer progression, that neuropilin-1 promotes survival and suppressive function of T_{reg}, and included a study providing new insights into the metabolic pathways that regulate T cell anti-tumor responses in ovarian cancer. Another study demonstrated that ovarian cancer patient monocytes are more tumoricidal when cultured with interferons than monocytes from matched controls, supporting a novel, innate, immune-based approach to immunotherapy of ovarian cancer.

In vitro models were represented by a 3D cell culture model for predicting the response to standard carboplatin chemotherapy, a 3D perfused bioreactor that allows the study of tumor biology and anti-tumor drug testing under physiological conditions, and models mimicking shear forces to improve our fundamental understanding of peritoneal metastasis and mechanotransduction. Therapeutics targeting ovarian cancer included the use of myxoma virus (MYXV) as a poxvirus that synergized with chemotherapy, aldehyde dehydrogenase 1A inhibitors increased LKB1 phosphorylation leading to AMP-activated protein kinase phosphorylation. McLean showed that the combination of inhibiting IL-6/LIF signaling with ruxolitinib with anti-estrogen therapy resulted in a synergistic decrease in ovarian cancer tumor cell viability. A new two-step targeting approach was presented that introduces non-natural targets (azide functional groups) in the tumors followed by the delivery of drug-loaded polymeric nanoparticles that are surface modified to have high affinity for these synthetic targets. Other pathways covered in the abstract session included apelin/APJ pathway in omental metastasis, role of TGF β 1/protein kinase C α /Twist1 signaling pathway in ovarian cancer metastasis, and the induction of insulin-like growth factor signaling by follicular fluid in fimbrial cells causing stemness, clonal expansion, and transformation. Yang-Hartwich presented data supporting the role of mutant p53 in promoting the initiation of HGSOC from fallopian tube precursors, while Rankin demonstrated that tumor-associated mesothelial cells promoted tumor invasion by increasing collagen deposition and remodeling.

CONCLUSIONS

The TME contributes to the 'hallmarks' of cancer and also shapes therapeutic responses and chemoresistance.^{4 6 10} It provides potential prognostic markers and therapeutic opportunities. Significant advances have been made in our understanding of the various components of the TME, including the cellular and acellular constituents. The 12th Biennial Ovarian Cancer Research Symposium reflected the increasing interest in the

TME of ovarian cancer and provided an excellent overview of the cutting-edge research going on in the ovarian cancer TME field, which provides renewed hope for our collective efforts to understand and eventually cure ovarian cancer. The identification of targetable molecular and cellular components in the TME will lead to the development of combination therapies that can simultaneously modulate TME and eliminate cancer cells to treat ovarian cancer more efficiently and effectively.¹¹ The new breakthroughs in the field of ovarian cancer TME that were presented at this meeting are leading the way in our fight against this deadly disease.

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REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. *CA Cancer J Clin* 2019;69:7–34.
2. Torre LA, Trabert B, DeSantis CE, et al. Ovarian cancer statistics, 2018. *CA Cancer J Clin* 2018;68:284–96.
3. Bowtell DD, Böhm S, Ahmed AA, et al. Rethinking ovarian cancer II: reducing mortality from high-grade serous ovarian cancer. *Nat Rev Cancer* 2015;15:668–79.
4. Hanahan D, Coussens LM. Accessories to the crime: functions of cells recruited to the tumor microenvironment. *Cancer Cell* 2012;21:309–22.
5. Mitra AK. Ovarian cancer metastasis: a unique mechanism of dissemination. In: *Tumor metastasis*. InTech, 2016: 43–58.
6. Dasari S, Fang Y, Mitra AK. Cancer associated fibroblasts: naughty neighbors that drive ovarian cancer progression. *Cancers* 2018;10:406.
7. Hansen JM, Coleman RL, Sood AK. Targeting the tumour microenvironment in ovarian cancer. *Eur J Cancer* 2016;56:131–43.
8. Odunsi K. Immunotherapy in ovarian cancer. *Ann Oncol* 2017;28(Suppl 8):viii1–7.
9. Hynes RO, Naba A. Overview of the matrisome - an inventory of extracellular matrix constituents and functions. *Cold Spring Harb Perspect Biol* 2012;4:a004903.
10. Nieman KM, Romero IL, Van Houten B, et al. Adipose tissue and adipocytes support tumorigenesis and metastasis. *Biochim Biophys Acta* 2013;1831:1533–41.
11. Pearce OMT, Delaine-Smith RM, Maniati E, et al. Deconstruction of a metastatic tumor microenvironment reveals a common matrix response in human cancers. *Cancer Discov* 2018;8:304–19.